PhD Synopsis

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SYNOPSIS FOR REGISTRATION OF Ph.D.

Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra State, India

(UGC recognised, NAAC Re-accredited 'B^{++}' grade state university)

Phytochemical Investigation and Pharmacological Evaluation of Some Ethnomedicinal Plant Parts for Neurobehavior and Nootropic Activity

Research Proposal submitted in partial fulfillment of registration to

Ph.D. Programme

In the subject

Pharmacology,

Faculty of Pharmaceutical Sciences and Technology

Submitted by

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ABSTRACT OF PROPOSED WORK PLAN

The present synopsis is for pharmacological evaluation of a selected plants part(s) for neurobehavior and neuroleptic activity in experimental animals. After proper literature survey the selected plants part(s) will be collected. The selected plants part(s) will be authenticated, cleaned, dried and powdered for extraction with different solvents (Non-polar to Polar). Preliminary phytochemical investigation will be carried out; extract will be subjected for fractionation. The fractionated plant part(s) extract will then be evaluated for acute toxicity study for the determination of safe dose (as per OECD guidelines) and screened for its preliminary effect neurobehavior and nootropic in animal models. The fractionated plant part(s) extract will then be evaluated for neurobehavioral and nootropic activities in experimentally induced neurobehavioral and nootropic conditions in animals. The obtained data will then statistically interpret, results will be analysed and the research findings will be disseminated by publications for the health care providers and lay public.

INTRODUCTION

The search for new molecules that act on the central nervous system (CNS) and that can be used for therapeutic purposes started with several studies in the 19th century. In fact, the first drugs used to treat pathologic conditions of the CNS were based on natural resources, specifically on plants. However, studies targeting plants with this type of bioactivity represent only a very small percentage of those investigations.

As per World Health Organization 1 in every 8 people or 970 million people in the world live with a mental disorder with anxiety and depressive disorders the most common (1). Mental disorders involve significant disturbances in thinking, emotional regulation, or behaviour. In 2020, the number of people living with anxiety and depressive disorders rose significantly because of the COVID-19 pandemic. Initial estimates show a 26% and 28% increase respectively for anxiety and major depressive disorders in just one year (2). While effective prevention and treatment options exist, most people with mental disorders do not have access to effective care.

Central nervous system (CNS) disorders represent a public health priority and demand significant scientific efforts for the development and study of new drugs and their possible beneficial effects. It has been estimated that CNS disorders result in death in one of every nine patients; these disorders include ictus, Parkinson's disease (PD), and Alzheimer's (AD) disease (3). There is a large amount of interest in natural compounds as, particularly in CNS disorders. In particular, various studies suggested that natural products can improve mood states, neuroinflammation, and neurodegenerative diseases (4,5).

Typically, treatment of emotional disorders includes a combination of drugs, as well as psychotherapy and family support. Dietary supplements, including herbal or plant based extracts, micronutrients, and food-based nutraceuticals are widely used in different neurological diseases, such as mood and cognitive function disorders (6)

Medicinal plants are of great value in the field of medicine and cure of diseases. Practical experience and several modern research studies have shown that therapy using plants is better than using synthetic chemicals by being safer besides having synergistic effect of their active ingredients and presence of certain minerals and salts. There is still large number of medicinal plants in which all active constituents have not yet been investigated even though their medicinal effect is established by folklore and traditional system of medicine. The traditional system of medicine has utilized herbal formulations for treating various human ailments. The side effects of drugs of chemical origin have shifted focus of

researchers towards natural therapeutics for curing health related problems in living beings. Although medicinal plant based herbal therapy is in existence from centuries but scientific validation is still a difficult challenge for developing herbal medicines. Multiple studies have shown that compounds and extracts derived from plants can cure or delay health related decline and extend lifespan and health span across a variety of species. Major hindrance in amalgamation of herbal medicine in modern medical practices is lack of scientific and clinical data proving their efficacy and safety. There is a need for conducting clinical research in herbal medicine, developing simple bioassays for biological standardization, pharmacological and toxicological evaluation and developing various animal models for toxicity and safety evaluation. It is also important to establish the active components from herbal extract.

REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT		
Plant	**CNS effect**	**Reference**
Acorus calamus	Ground up rhizomes are used for nervous disorders. They also have been reported to be ground up and mixed with *dagga* (*Cannabis sativa* L.) for use in tonics and stimulants. Rhizomes and roots have sedative and analgesic properties and several other properties not related to CNS activity.	Stafford et al. (2008) (7)
Anemopaegma arvense	Unlike benzodiazepine agents, the anxiolytic flavonoid does not induce sedation while producing antianxiety activity. The tonic has shown aphrodisiac and memory enhancer effects, and the ability to improve mental fatigue has been described	Hui et al. (2002), Liao et al. (2003), Mendes and Carlini (2007), Rodrigues et al. (2008) (8,9,10,11)
Bacopa monniera	Largely used in India, *Bacopa monniera* is valued as a revitalizing herb that strengthens nervous function and memory. The extracts nootropic action or antiamnestic effect has been confirmed, as have their antistress activity, anticonvulsive action, antidepressant activity, and anxiolytic effects.	Kishore and Singh (2005), Kumar (2006), Rai et al. (2003), Sairam et al. (2002), Shankar and Singh (2000), Singh et al. (1996) (12,13,14,15,16,17)
Convolvulus pluricaulis	An herb commonly used in India for hundreds of years for nervous disorders, it is reported to produce a feeling of peace and calm and reduce stress, anxiety, and mental fatigue.	Kumar 2006 (12)
Ficus platyphylla	Aqueous extracts of *Ficus platyphylla* bark have been used traditionally to calm agitated people. The plant has also been studied for behavioural effects in psychotic patients. The results have revealed the psychotropic value of the plant's extracts.	Gamaniel et al. (2000), Vongtau et al. (2005) (18,19)

OBJECTIVES OF RESEARCH

AIM - "Phytochemical Investigation and Pharmacological Evaluation of Some Ethnomedicinal Plant Parts for Neurobehavior and Nootropic Activity"

OBJECTIVES -

i) To extract plant part (s) using suitable solvent (s)

ii) To investigate and standardization of some selected medicinal plant extracts/fractions.

iii) To studies safe dose of plant extracts

iv) To evaluate preclinical pharmacological studies of plant extracts/fraction for Neurobehavioral and Nootropic activity.

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METHODOLOGY TO BE ADOPTED

1. Plant Material:

Selected plant parts will be collected from local known sources and get authenticated from expert Botanist of the region.

2. Processing of crude drug:

Collected crude drug subjected to processing such as drying, grinding, sieving etc as per official standards.

3. Pharmacognostic Evaluation of plant material:

Parts of selected plants subjected to Pharmacognostic evaluation viz;

a) Morphological Evaluation

b) Microscopical evaluation and powder characteristics

4. Extraction and fractionation of Plant material:

Powder form of selected individual plant material subjected to extraction/fractionation. The choice of solvent for extraction/fractionation and extraction/fractionation methods decided according to chemical constituents of selected plant's part and nature of chemical constituents.

Any one or two methods of Extraction/fractionation will be used including Soxhlet's method separation funnel method/ chromatographic techniques/fractional distillation.

Fractionation of selected plants:

Fractions of plant extract will be carried out successively by sonication method with solvents using ultrasonicator. All the fractions were collected, filtered through Whatman filter, concentrated and stored in tight desiccator further physical properties like color of fractions, its consistency, net weight and % yield.

5. Phytochemical Evaluation of plant extracts/fractions:

Phytochemical examinations carried out for all the extracts as per the standard methods. It includes estimation for extractive value, acid &water soluble ash, LOD, presence of foreign matter, heavy metal content, microbial contamination etc. Qualitative analysis carried out for detection of phytoconstituents and later confirmed by using suitable chromatographic technique.

6. Pharmacological Screening of plant extracts:

Selected plants partextracts either individually or in combination evaluated for-

I. In Vitro-Antioxidant study

In vitro antioxidant activities for the extracts will be carried out using the following methods.

1. DPPH assay

2. Hydrogen peroxide scavenging assay.

3. Nitric oxide scavenging activity

4. Ferric chloride scavenging activity

Any two or three methods from above will be used for Anti-oxidant activity

II. Safe dose calculation:

1. Safe dose calculation of plant extracts for the purpose of use of extracts for efficacy evaluation carried out as per OECD Guidelines for 'Acute Toxicity study: Class Method (423).

III. Study on suitable animal models for Neurobehavioral and Nootropic activity.

A. Anti-anxiety Activity

1. **Light and Dark Model**
2. **Elevated Plus Maze**

B. Anti-Depressant Activity

1. **Tail Suspension Method**
2. **Despair swim Test**

C. Anti-aggressive Activity

D. Memory Enhancing Activity

1. **Morris water Maze**
2. **Radial Arm Maze**

From above one or two models will be performed.

7. Interpretation of data and its presentation:

- Data collected that for Pharmacognostic evaluation of selected plants part presented in tabular form and microscopic observation presented as figures of TS & for powder characteristics.
- Qualitative evaluation of plant extracts for the content of phytoconstituents presented as either present (+ve) or absent (-ve) and presence of phytoconstituents confirmed by TLC by comparing with Rf value of standard. Fractioned extracts subjected for phytoconstituents characterization by suitable methods.
- Safe dose calculated as 1/10 dose of LD_{50} value of plant extracts/fractions
- All the data related to efficacy of extracts/fractions of selected plant's part subjected to statistical analysis like student's t test, ANOVA etc. to find level of significance between extracts/fractions and between extract/fractions to control/positive control data. Such data will also be presented as chart diagram.

Significance of the study

a) Now a day the world trend is toward the utilization of medicinal plants to treat various ailments.

b) Several plants possess potential therapeutic properties and the claims of many medicinal plants used in traditional medicine are now supported by scientific study.

c) Pharmacognostic studies ensure plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.

d) The problem of Plant-based treatment is the lack of definite and complete information about the composition of extracts.

e) Evidence for the potential therapeutic effects of selected herbs is generally provided based on experiments demonstrating a biological activity, thus pharmacological evaluation of medicinal plant confirms its potency.

f) Plant derived remedies need a powerful and deep assessment of their pharmacological qualities and safety that actually can be realized by pharmacological evaluation.

g) The side effects and expensiveness issues associated with synthetic drugs have developed interest for research in to medicinal plants which are generally without side effect, especially those belonging to traditional system of medicine.

PROPOSED WORK PLAN

1. **Literature review**
2. **Collection, Identification and authentication of selected plant material**
3. **Processing of Crude drugs**
4. **Pharmacognostic evaluation of plant materials**
5. **Extraction of selected plant materials-**
    a) Selection of Extraction method
    b) Selection of Solvent
    c) Selection of fractionation method
6. **Phytochemical Screening**
    a) Phytochemical screening of extract for
        i) Extractive Value & Ash content
        ii) Acid &Water soluble ash
        iii) Foreign matter
        iv) LOD
        v) Heavy Metal Content
        vi) Microbial Contamination
    b) Phytochemical Qualitative analysis for presence of -
        i) Carbohydrates, tannins, saponins, alkaloids, triterpenoids, glycosides, lipids, flavonoids, phenols, proteins, steroids etc in plant extracts.
    c) TLC fingerprinting
    d) Standardization of extracts for
        a. Total polyphenols,
        b. Total flavonoid,
        c. Total saponin
7. **Pharmacological Screening of selected plant extracts for-**
        i) In Vitro-Antioxidant study
        ii) Safe dose studies
        iii) Suitable In-Vivo/In-Vitro models for neurobehavioral and nootropic activity
8. **Interpretation of data and its presentation**

Extensive literature survey reveals numbers of medicinal plants are claimed in traditional system for its effectiveness in neurobehavioral and nootropic activity or so many plants just evaluated for its neurobehavioral and nootropic Activity. Accordingly for present study following medicinal plants are shortlisted for further literature survey so as to select two to three medicinal plants for study

YEAR WISE PLAN OF WORK AND TARGET TO ACHIEVE

Present study is designed in such a way that it will come to a conclusive outcome within 03 years from the date of allocation. The year wise plan includes**,**

YEAR 01

- Literature survey;
- Collection, identification and authentication of plant parts(s)
- Processing of authenticated plant parts(s) such as drying, segregation, grinding, sieving etc. to make it suitable for extraction;
- Preliminary screening, physical evaluation of powdered drug.

YEAR 02

- Extraction of the plant parts(s) by selected and optimized method of extraction;
- Assessment for presence of important phytoconstituents in extracts/ fractions by different qualitative and quantitative methods of evaluation as well as standardization;
- *In vitro* pharmacological screening of different extracts for neurobehavioral and nootropic activity.

YEAR 03

- *In vivo* pharmacological screening of different extracts/fractions, for neurobehavioral and nootropic activity using suitable screening methods;
- Bioactivity guided fractionation,
- Pharmacological screening of bio active phytoconstituents using selected pharmacological evaluation methods;
- Compilation and interpretation of data;
- Communication of research and/or review/research articles and thesis writing.

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